- a confocal optical system including signal generating means cooperating with

- withdrawing means, connected to said receptor means, said withdrawing means is controlled mechanically, optically or electrically.

107. A device for performing the method according to claim 87 comprising

- a sample compartment and a receptor compartment connected by

- receptor means;

- a confocal optical system including signal generating means cooperating with

- withdrawing means, connected to said receptor means, said withdrawing means is controlled mechanically, optically or electrically.

F5 End

REMARKS

Claims 68-107, presented hereby, are pending.

The subject matter of claim 68 is disclosed, e.g., on page 18 (screening of pharmacologically active substances), page 2, page 10 (use of a confocal optical system), page 8 (withdrawal on basis of the signal). The subject matter of claims 69-71 is found at the bottom of page 14 and the top of page 15 of the specification. The subject matter of claims 72 and 73 can be found in the specification at the bottom of page 8, and the subject matter of claim 74 at page 18.

The invention of the present claims is defined by (i) use of small measuring volume elements (which are typically generated using confocal microscopy) in conjunction with (ii) screening for pharmacologically active substances or pharmacologically active target molecules.

In the course of preparing the present reply, Applicants noticed some clerical errors in the specification, which are corrected hereby. The requisite marked-up version is attached, hereto.

On page 6, the smallest aperture of the pores is defined by a size D according to the formula 100 $\mu m \leq D \leq 0.1 \mu m$.

At several places in the application (e.g. page 14, bottom; page 15, bottom) the term "object plane" is replaced by the term "image plane". It is well known by the skilled person that, e.g., the pinhole aperture, multiarray detector, and the optical waveguides within confocal optical systems are placed in the "image plane," not in the "object plane". The term "object plane" is an obvious error, as it would imply that the pinhole apertures, multiarray detectors, and the optical waveguides are placed inside the sample, which cannot be the for case technical reasons unequivocally.

Former claim 33 was rejected under 35 U.S.C. § 102 (a) for being allegedly anticipated by Eigen et al. (Proc. Natl. Acad. Sci. USA, 91, 5740-5747 (1994)). Reconsideration is requested.

To overcome the alleged anticipation by Eigen et al. under §102(a), the verified translations of the two German priority documents P 44 22 313 and P 44 22 290 are submitted herewith. The effective date of Eigen et al. for purposes of prior art is June 27, 1994. Since the two priority documents have respective filing dates of June 17 and June 25, 1994, Eigen is antedated by the priority documents and, as such, not available as prior art.

Claims 33-46, 53-59 and 67 were rejected under 35 USC 112, ¶1, for allegedly lacking enablement, especially, with regard to the disclosure of how the generated signal (1) defines the volume element, (2) determines the time for withdrawal of the volume element, and (3) controls the withdrawal as set forth in claim 33.

Applicants submit that the disclosure in the instant application mentioned in the following provides sufficient teachings to satisfy the enablement requirements of §112, ¶1.

Different examples are described in the specification, giving information on how to define the volume element and the time to transfer the volume element. Information is e.g. provided in the specification on page 2 (bottom), page 5 (middle), page 8, and page 10. A very detailed description of one example for the identification and withdrawal of microorganisms is shown in Fig. 4. Bacteria are induced to express certain biological expression products. Such expression products can be detected through the use of marker-molecules, such as fluorescently labeled molecules which bind to the expression products. Such a binding interaction can be detected by illuminating the sample with a laser and analyzing the emitted fluorescence. A very suitable analysis method is fluorescence correlation spectroscopy (FCS). Unbound fluorescently labeled molecules are capable of diffusing very fast through the measuring volume in comparison with those molecules bound to the expression product. During the time the bound and unbound molecules are spotted by the laserlight they produce a fluctuation in the temporarily and spatially resolved fluorescence signal which provides a basis for the identification of molecules to be withdrawn. The output of the detector is processed and a sorting decision is taken on the basis of measured parameter values, such as the diffusion time of the

molecules. In the example at issue, the decision to remove molecules bound to expression products activates microdrop pumps which withdraw the molecules bound to the expression product from the sample.

The presently claimed invention is based on analyzing extremely small measuring volumes as a part of a larger, surrounding sample volume. Such measuring volumes can be defined by confocal illumination. Using these highly sensitive and precise analysis methods the space of a particular component, such as the bound molecule, at a given time can be very precisely determined.

As described on page 8 of the specification, a hardware/software-coupled on-line operating system triggers the withdrawal time through the measured signal, e.g., a specific fluorescence signal or the diffusion time of molecules.

To satisfying the requirements of enablement under §112, ¶1, the specification must teach the skilled person how to practice the invention, with any gaps in the teachings provided being filled by the knowledge possessed by such skilled person. Taking the aforementioned disclosure of the specification into account, the knowledge of the person skilled in the art fills in any gaps.

For instance, US 4,887,721 (Martin et al.), of record, in Fig. 1 and the corresponding description in column 3, disclose that one or several detector systems measure parameters of interest of the particles under study. The output of the detector is processed by electronic circuitry and a sorting decision is taken on the basis of measured parameter values. Martin et al. utilized for withdrawal of particles a deflection beam. The system driver of the detector system controls via an intensity modulator the intensity of the deflection beam. Depending on the state of this deflection

beam a particle can be withdrawn from the sample. Accordingly, the person skilled in the art would have known how to connect the detection system used to identify particles to be withdrawn with the withdrawal system.

Claims 33-46, 53-59 and 67 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Martin et al., Ashkin et al., North et al., Gohde et al., and Weber et al. The rejection is not applicable agains the present claims.

Two new independent claims define alternatives of the method of the instant invention. The first method relates to the screening of substances for their pharmacological activity. In an example, a putative pharmacologically active substance binds to a pharmacological target molecule, which is e.g. bound to the surface of a cell or vesicle. The interaction is detected by the confocal optical system and the signal is used to trigger the withdrawal process. In a preferred embodiment, the method relates to a so-called competitive screening assay. A ligand is capable of binding to a pharmacological target molecule is added to the sample. If the substance introduced to the sample is pharmacologically active with regard to such target molecule, it competes with the ligand for the binding to the target molecule. In case of a strong interaction between such substance and such target molecule the labeled ligand is displaced by the unlabeled substance. The interaction is then detected again triggering the withdrawal process.

The second alternative of the method claimed is used for the identification of pharmacological target molecules by using labeled, known pharmacologically active substances. Known drugs might interact with different target molecules such as receptor molecules. In case the

substance binds to the target molecule this interaction is an indication for a potential pharmaceutical relevance of the target molecule. The interaction can be detected and the signal used to trigger the withdrawal process (see, e.g., page 18).

Applicants submit that the asserted combination of references neither teaches nor suggests either the first or second alternative of the method presently claimed, as discussed above.

Martin et al. describe an extraction system, wherein a first laser defines an optical path and which is effective to propel the particles through a measurement volume. A probe laser beam interrogates the components to identify predetermined characteristics and activates a second laser beam through an output signal relating to predetermined characteristics. The extraction of a component is achieved by a second laser beam intersecting the first laser beam in combination with a video system and a computer as optical analytical system.

Ashkin et al. describe optical traps for the manipulation of biological components. For operation the optical trap is introduced into a microscope while being viewed by a video camera. Separation of components from a sample and their introduction into another is accomplished by manipulating them into a liquid-filled hollow-glass fibre.

North et al. describe a driver circuitry driven tube to remove components. A fluorescence meter is used as analytical method. An operator receives a signal from the analysis portion relative to one or more components characteristics and selectively aligns the catcher tube in communication with the analysis portion.

Goehde et al. describe a system, wherein after fluorescence analysis a cell is sorted due to a piezoelectric transducer producing a pressure pulse. Particles to be selected pass a measurement location in which particles to be selected trigger a signal by a sensing device. Downstream the pressure pulse is produced in response to the signal. Particles are excited to fluorescence, e.g., using a microscope-incident light arrangement through a microscope objective.

Weber et al. describe withdrawal systems for the extraction of cells using video and computers.

None of the cited references relates to "a method for screening substances for their pharmacological activity" or "a method for identifying pharmacologically active target molecules", as presently claimed. Additionally, none of the cited references teaches the use of confocal optical systems for screening or target molecule identification, as used in accordance with the presently claimed inventikon. Because none of the references contains such a teaching, the asserted combination cannot render the present claims unpatentable under §103(a).

The present application shows the shortcomings of the instruments and methods applied in the cited references. For example, assume it to be desirable to determine the enrichment of smallest amounts of a fluorescent dye bound to a substance to be screened on the surface of a suspended target molecule, which is bound to a cell or vesicle, and use the signals obtained for triggering the withdrawal process. It would, then, be desirable to withdraw those cells or vesicles with target molecules which have bound to such fluorescently labeled substances. Due to the small measuring volumes, large amounts of fluorescent dye can be present in the surrounding fluid without disturbing

the identification of the cells or vesicles to be withdrawn. The differentiation of fluorescence signals allows the use of the method of the presently claimed invention for screening of pharmacologically active substances against a variety of target molecules.

On the other hand, by using integrated signals as described in the prior art, the detection signal could be dominated by the fluorescence signals produced in the surrounding liquid. The specific contribution of the substances on the surface of the cells or vesicles would be too small to receive reliable results. Statistical distribution of substance and/or bead properties, or the different position of the cells or vesicles when passing the measurement volume, produce significantly larger variations in the fluorescence signal and dominate the variation in the fluorescence signal caused through binding of fluorescent dye on the surface of the cells or vesicles. Reliable information about the particle to be withdrawn cannot be obtained this way, at least not in the time typically allowed in pharmacological high throughput screening applications.

However, the use of confocal analytical methods applying small measuring volume elementsin accordance with the teachings of the presently claimed invention allows the fluorescent signal of interest on the surface of the cell or vesicle to be determined separately and with high accuracy. In case the confocal measuring volume element is positioned on the surface of the cell or vesicle, contributions of the fluorescence from the surroundings, as explained above, can be eliminated.

The method of the presently claimed invention solves the problem in the art of identification of pharmacologically active substances and target molecules. The presently claimed invention allows

the separation of such substances or pharmacological target molecules by obtaining, e.g., a much better signal to noise ratio than in the conventional systems of the cited references.

Favorable action is requested.

Respectfully submitted,

JACOBSON HOLMAN PLLC

By: _____
William E. Player
Reg. No.: 31,409

The Jenifer Building
400 Seventh Street, N.W.
Suite 600
Washington, D.C. 20004
(202) 638-6666
Atty Dkt. No.: P60752US0
Date: March 18, 2003
WEP/rdt
R:\rthomas\2003\MARCH\P60752US0-Amdt.wpd

Marked up version of amendments

IN THE SPECIFICATION:

Page 6, please delete the first paragraph and replace it with the following paragraph:

The method permits the withdrawal of one or a few components of a system, such as molecules, molecular complexes, vesicles, micelles, cells, optionally embedded in an associated volume element (withdrawal volume), V (10^{-9} 1 ≥ V ≥ 10^{-18} 1). This volume element is part of a larger volume of an environment which contains the small components to be withdrawn (sample volume). The withdrawal is effected by transferring the component or components to another environment wherein space and time of the withdrawal are determined by analytical signal which is correlated with the small component to be withdrawn. The analytical methods which may be used are those by which the molecular contents of smallest volume elements (10^{-14} 1) can be analyzed as described in the International Application of Rigler et al., PCT/EP 94/00117. The sample volume is connected with a receptor means through a pore of a capillary or a pore of a membrane wall whose smallest aperture D is given by 100 μ[(] m ≤ D [(] ≤ [0,1] 0.1 μm.

Page 14, please delete the fourth paragraph and replace it with the following paragraph:

Extended arrays of small volume elements can be illuminated by using holographic grids. According to the invention, the measuring volumes are measured confocally for fluorescence properties of molecules contained therein by using a multitude of pinhole apertures in the [object plane] image plane, by positioning multiarray detector elements in the [object plane] image plane,

or by using optical fiber bundles to which the light is coupled in the [object plane] image plane and transferred to photon detectors.

Page 14, please delete the fifth paragraph (which bridges over to page 15) and replace it with the following paragraph:

In the highly parallelized illumination of small volume elements, there is the problem of registration of the emitted fluorescence signals from the individual volume elements. In the patent application PCT/EP 04/00117, it is reported that it is possible to illuminate small space elements in parallel and to focus the respective fluorescence signals individually on multiarray detectors by using confocal pinhole aperture systems in the [object plane] image plane, or to couple the signals into optical waveguides at the position and in lieu of the pinhole apertures and to guide them onto detector elements, or to position the multiarray detectors themselves in lieu of and at the position of the pinhole apertures. There is also described the possibility to illuminate a larger volume element and to combine it with the above described confocal, parallel focussing of small subvolume elements.

Page 15, please delete the fourth paragraph and replace it with the following paragraph:

According to the invention, at least two measuring volumes in common or assembled in groups are focussed confocally onto at least one detector element of a photon-registrating measuring element in the [object plane] image plane in the signal registration.